PATENT
Attorney Docket No.: 27373/39055B

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: Wang *et al.*

Application No. 09/748,710

Filed: December 22, 2000

For: METHOD FOR GENERATION OF LONGER CDNA FRAGMENTS FROM SAGE TAGS FOR GENE IDENTIFICATION

Group Art Unit: 1637

Examiner: Joyce Tung

I hereby certify that this correspondence is being deposited with the U.S. Postal Service with sufficient postage as First Class Mail, in an envelope addressed to: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450, on the date shown below.

Date: August 26, 2003

William K. Merkel

DECLARATION OF SAN MING WANG, PH.D., UNDER 37 C.F.R. §1.131

I, San Ming Wang, Ph.D., state that,

1. I am an inventor of the subject matter claimed in the above-identified application, which claims priority to U.S. Provisional Application Nos.: 60/174,391 and 60/173,617, filed January 3, 2000, and December 29, 1999, respectively. I am making this declaration to provide evidence that the subject matter claimed in the above-identified application was completed in the United States at a date at least prior to July 19, 1999, which is the date of acceptance of van den Berg, et al., Nucleic Acids Research, 1999, Vol. 27(17), pages i-iii, cited by the Examiner in an Office Action mailed March 26, 2003.

2. To establish the date of completion of the invention, copies of pages from my laboratory notebook are attached as Exhibit A. All dates appearing on these laboratory notebook pages have been redacted.

3. The pages from my laboratory notebook establish the following facts:

(a) Information from SAGE tags alone do not provide sufficient information for gene identification;

(b) The solution to the problems associated with SAGE tags was to extend the SAGE tag sequence to the 3' end of the cDNA;

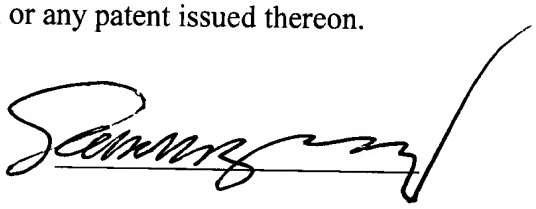
(c) The longer 3' cDNA could be generated using a SAGE tag as a primer in an extension and/or amplification reaction(s);

(d) One amplification reaction useful in solving the SAGE tag problem is PCR, using a SAGE tag as a sense primer and a set of universal primers consisting of oligo (dT) with A, G, CA, CG, CC anchors as antisense primers, and using the cDNA sample used in SAGE tag collection as a substrate;

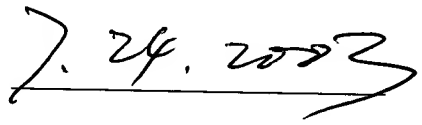
4. These laboratory notebook pages confirm that the subject matter of the pending claims in the application was completed at least prior to July 19, 1999.

5. This declaration is submitted in response to a non-final Office Action.

6. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true and, further, that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.



San Ming Wang, M.D.



Date

EXHIBIT A

select candidate genes from different classes based on the tag sequences from Lin Zhang, John Hopkins.

- Confirm the matched genes from tag seq. by Blastn parameter set: expect: 1000, Cutoff: 60. sequences aligned show many aligned genes in many cases to define the most likely ones, it needs to stress:

  a. 5' add CATG

  e.g. 5' CATG XXXXXXXXXX
  tag

  b. the matched sequences should be located in the 3' last NlaIII site of the sequence

  c. confirm the right gene by ~~using~~ generally large templates based on the tag sequence. This is not available in SAGE technique. I designed a system to stress this issue. If solved, it will largely improve the reality of ~~SAGE~~ gene identity in SAGE.

REDACTED

- based on Taq seq. design 5' seq (sense primer, plus 6 bases tail to maintain the stringency
- use anchored primer as 3' antisense primer to select 3' subpopulation for PCR. IMPORTANT!
- PCR amplify cDNA NEW IDEA.
- the cDNA should be pre-digested by NlaIII.

The possible outcome would be

- specific temp amplified because of the 5' sense primer / 3' anchored primer gives exponential amplification, others will only be ~~double~~ increased, 1, 2, 3, ...

potential problem:

- amplification efficiency lower down to 3' extension consume large portion of substrants
- specificity ?

~ anchored dT dy. It is know well + Taq pol precisely distinguish base anchored primer CR reaction.

REDACTED model system to confirm the possibility.

genes β-Actin S2/AS S2/mixture of aT, A/G/C

HSC 70

HSP 75

HSP 78

	1x	4x
H2O	31	124
MgCl2	5	20
buffer	5	20
dNTP	5	20
Taq	1	4
cDNA (aT12-18 HL60 mRNA)	1	4

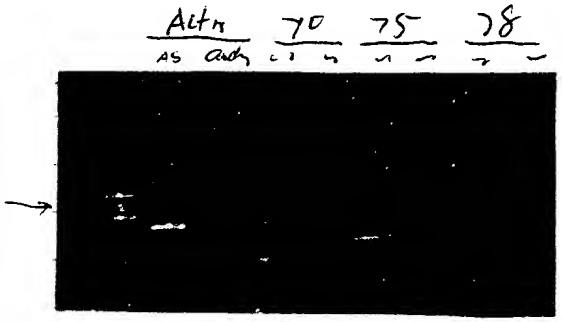
Sense L

4μ 4μ 4μ 4μ

1 (actin) 1 (70) 1 (75) 1 (78)

2μ 2μ 2μ 2μ 2μ 2μ 2μ 2μ

antisense 0.5 (AS) 0.5 (mix each aT) " " " " " "



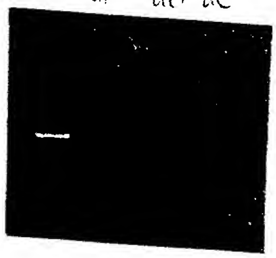
95 20"
50 20" ×5 → 60 20" } ×25
72 20" 72 20"

Conclusion. the mixture of a-chased primers doesn't provid

The possible reason for failure to amplification may be that the mixture of anchor dT disturbed the efficient amplification; Try to use separated anchor-dT as 3' primer. use Actin as model

	2X
H_2O	62
$MgCl_2$	10
buffer	10
dNTP	10
Taq	2
cDNA	2
sense (actin)	2

25	25	25	25
0.5 (AS)	0.5 (dAT_n)	0.5 (dGT_n)	0.5 (dCT_n)



β-actin mRNA end (HSAC07)

…AGTG CACAC CTTA$_n$

A TTTTT…TTT ($dA3T_n$)

Conclusion (1) dA anchor as 3' O.K.

REDACTED

Improve:

- increase 3' anchor primer amount
- decrease first 5 cycles in PCR to favor 3' primer bind to temp. then shift to 60°C for annealing
- increase cycle number to 40 ✓ Actin

	8x
MgCl2	40
Buffer	40
dNTP	40
Taq	8
cDNA	8
Sense	8

8 × 15

q → dA, dG, dC

dATn
dTTn
dCTn

ef1-α
ribosomal
(3)α
S24

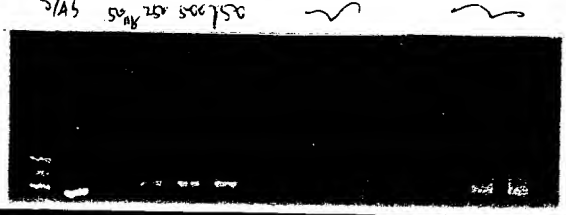
X03743
M69013
U26726

AA514262
AI193316
S28
AA676952
AA617972
AJ223473
D25786

antisense	1	5	10	15	15	10	15	1	5	10	15		
	50ng	250ng	500ng	750ng									
H2O	14	10	5	—	14	10	5	—	14	10	5	—	

25 nl

94°C 20" / 42°C 20" / 72°C 20" } ×5 → 94°C 20" / 60°C 20" / 72°C 20" } ×30



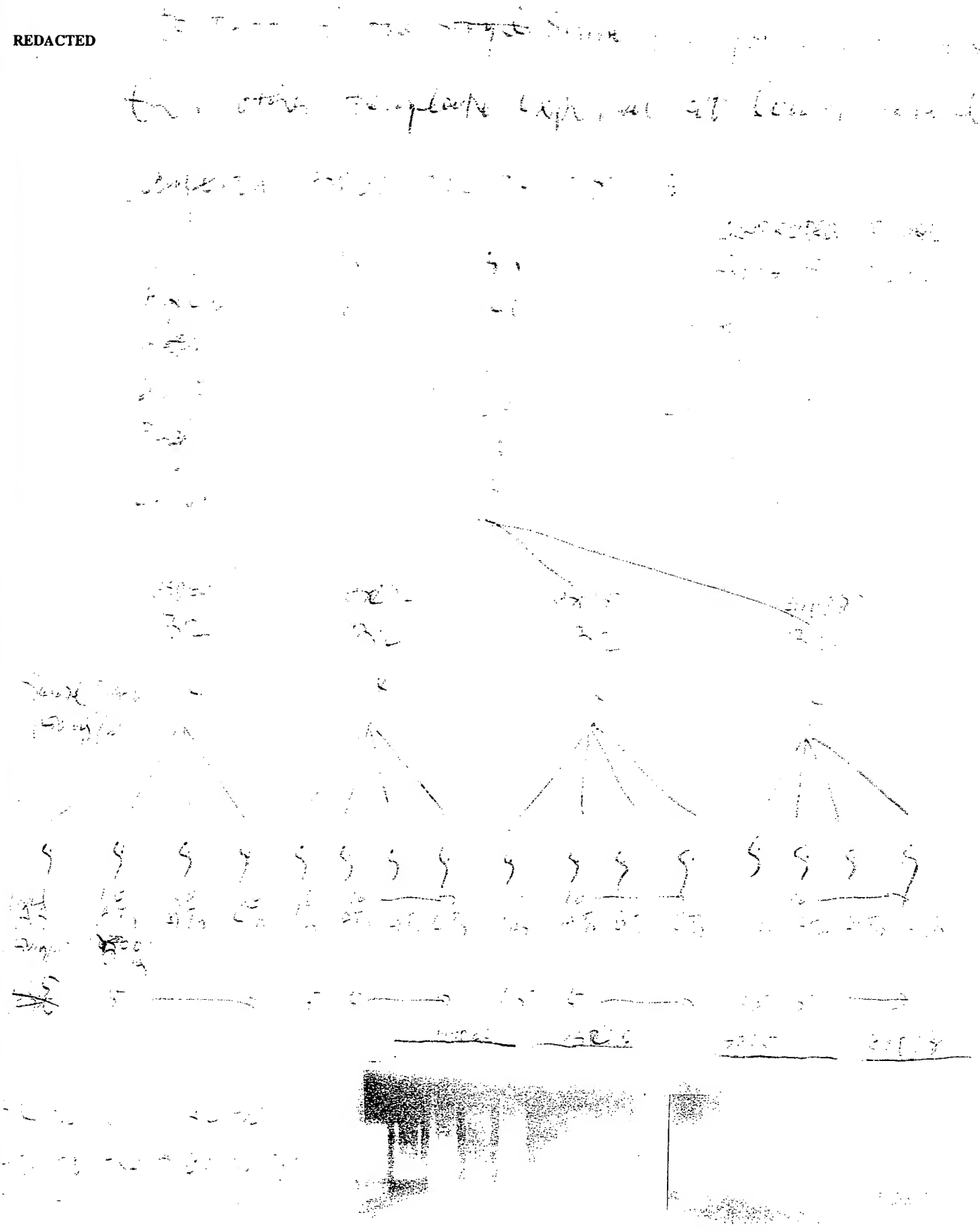
Conclusion:

① increase anchor primer amount did increase the amplification eff.

② CTn still generated diffused

In principle, this strategy should work! Can be use

REDACTED

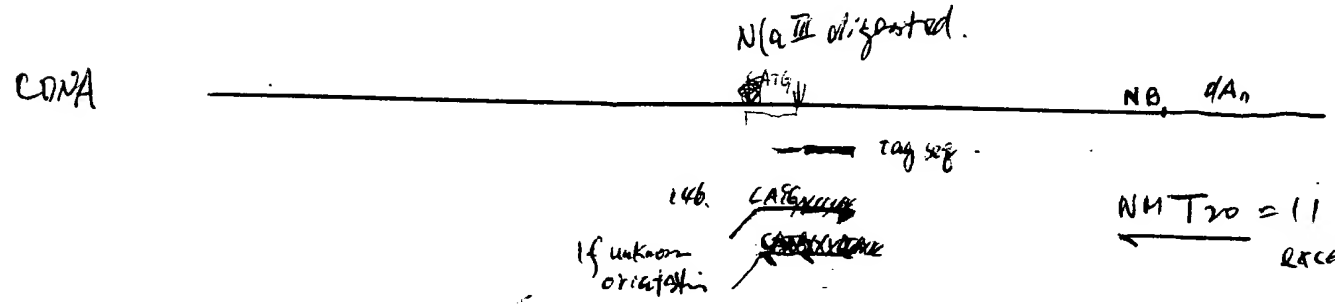


* also, the cDNA should be generated with anchor set not oligodT which creates background in PCR.

It seems that one base anchored dT primers may not generate enough specificity for particular temp, due to the complicated cDNA pool composition, or different members in the same family like HSP70s

TO increase the specificity, it may be useful to use 2 base anchored dT_n set as 3' antisense primer

The complete set of sense / antisense will be



- To identify each ~~temp~~ tag gene, the total reaction will be 11 PCR reaction with Tag seq as Sense (+ 6 base 5' to
- If negative, reversed Tag sequence will be used as the Sense primer in case the original Tag was generated in 3'-5 direction.
- in this way, the specific 3' fragment will be generated sequenced, and match to database.

next. try repeat exp. with anchor primer generated cDNA → {one base anchor 5' 5